

## Sperm Development and Ultrastructure of Mature Spermatozoa of *Megalyra* (Hymenoptera: Megalyroidea)

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**Abstract.**—Sperm ultrastructure and aspects of spermiogenesis are described for the first time for a member of the hymenopteran superfamily Megalyroidea, the parasitic wasp, *Megalyra fasciipennis* Westwood. The Megalyroidea are a poorly-known, putatively ancient group of the Apocrita (wasp-waisted wasps), and they are the first member of the Evaniomorpha group of superfamilies (*sensu* Rasnitsyn 1988) whose sperm have been investigated in detail. Therefore their spermatology might provide clues as to the groundplan for the higher Hymenoptera.

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The Megalyroidea are among the more elusive and least well known of the parasitic wasps, currently placed in the informal 'Evaniomorpha' group of superfamilies as defined by Rasnitsyn (1988) which comprises the Ceraphronoidea, Evanoidea s.l., Megalyroidea, Stephanoidea and Trigonaloidea. However, few characters are known that unite this morphologically and biologically heterogeneous group, and its monophyly is only weakly supported (Ronquist et al. 1999). How the Megalyroidea are related to the other apocritan (wasp-waisted) Hymenoptera is therefore not well understood, and several features suggest that they may be one of the most basal groups, forming a transition between the parasitic sawfly family Orussidae and the other parasitic wasps (see Gibson 1985; Shaw 1990; Heraty et al. 1994; Downton & Austin 1994; Vilhelmsen 1997). Few species are known and most are known from only one or a few specimens in collections (Shaw 1990). They are idiobiont ectoparasitoids of concealed hosts, probably the majority attacking the wood-boring larvae of large beetles including the pest cerambycid, *Phoracantha*

(Shaw 1990; Austin *et al.* 1994). Introductions of one Australian species, *Megalyra fasciipennis* Westwood, into South Africa in an attempt at biological control of *Phoracantha* has resulted in a population of the parasitoid that in some years becomes numerous enough to permit guaranteed and relatively straightforward collecting, and it was in one of those years that material was obtained for spermatological investigation. This study provides the first spermatological information on the Megalyroidea, and is also the first detailed ultrastructural investigation of sperm for any of the 'Evaniomorpha' superfamilies. The work was carried out to provide basic ultrastructural information on sperm development in this rarely studied group, and to see if comparison with other Apocrita can provide phylogenetic information.

That sperm ultrastructure might provide new character systems for resolving relationships within the Hymenoptera was muted by Jamieson (1987), it was not until the preliminary comparative survey of non-aculeates by Quicke et al. (1992) that some of the wealth of characters they possess became apparent. But, while a grow-

ing number of superfamilies of Hymenoptera have had at least one included species examined in detail for sperm ultrastructure (see for example, Thompson & Blum 1967; Lensky et al. 1979; Lingmei & Dunsu 1987; Wheeler et al. 1990; Newman & Quicke 1998, 1999a,b) there are a number of important and phylogenetically significant groups for which nothing is known, for example, Orussoidea, Stephanoidea, Trigonaloida and even the Evanoidea, Ceraphronoidea, Cynipoidea and Platygastroidea, the last four of which are common and easy to obtain alive. It is hoped therefore that the present work will help encourage others to investigate the spermatology of these taxa in order to add to the body of phylogenetic information for resolving higher relationships within the order.

## MATERIAL AND METHODS

Recently eclosed adult male *Megalyra fasciipennis* Westwood were collected in South Africa in September 1998 and transported by air to the U.K., where testes and vas deferens were dissected under 2% glutaraldehyde in phosphate buffered saline (pH 7.2), fixed for two hours, then transferred to 2% osmium tetroxide in cacodylate buffer (pH 7.2) for a further 2 hr fixation. After two buffer washes, tissue was dehydrated to 50% ethanol and then contrasted with a saturated solution of uranyl acetate in 50% ethanol prior to complete dehydration, embedding in Epon resin and polymerisation overnight. Large silver sections were picked up on high resolution grids and contrasted with uranyl acetate and lead citrate.

## RESULTS

Mature sperm of *Megalyra fasciipennis* collected from the vas deferens were not formed into spermatodesmata. The individual sperm ranged in length from 160 to 200  $\mu\text{m}$ , though most were close to 180  $\mu\text{m}$  of which the head (acrosome plus nucleus) constituted approximately 17%. The head

was not much wider than the tail at its widest and could be seen to taper from the posterior of the nucleus to the tip of the acrosome.

The mature sperm of *Megalyra* (Fig. 1), illustrate all of the organelles so far described in the sperm of other parasitic wasps, i.e. axoneme, mitochondrial derivatives, deltoid bodies, acrosome and nucleus. In the testes cysts are found with sperm at many different stages of development. Primary and secondary spermatocytes are found with the latter in a syncytium formed by incomplete cytogenesis following the earlier mitotic division (Fig. 2). The structural features are as follows:

**Axoneme.**—The tail portion of the mature sperm contains a single axoneme with a  $9 + 9 + 2$  arrangement of microtubules for most of its length (Fig. 3). There are well developed accessory structures, particularly linking the central pair of microtubules (Fig. 3, arrowed).

The axoneme develops from an electron dense structure which becomes positioned adjacent to the nucleus defining the posterior pole of the nucleus (Fig. 4). This structure, often referred to as a ring centriole (Fig. 5), contains the basal body of the axoneme, from which the axoneme elongates. However, during spermiogenesis, many sectioned cells are found with between two and four axoneme profiles (Fig. 6). Usually one or more of the profiles exhibits a loss of structural integrity (arrowed Fig. 6) often appearing with less than the full complement of microtubules. It is not exactly clear what these represent and the possibility that they are degenerating cells cannot be ruled out, though their prevalence and the presence in each of at least one apparently perfect axoneme profile suggest that they are a normal developmental stage. No mature sperm with multiple tails have been found.

**Mitochondrial derivatives and centriolar adjunct.**—The mature sperm has two differently sized crystalloid mitochondrial de-

rivatives (Figs. 1 & 3), which run for the length of the tail. The larger of the two mitochondrial derivatives also runs into the head of the sperm, parallel to the nucleus (Fig. 7). In transverse section (Fig. 8) it can be seen that the mitochondrial derivative can occupy a greater proportion of the shaft area than the nucleus itself (Fig. 8, *arrowed*).

The mitochondrial derivatives are derived from a Nebenkern, the product of the fusion of large numbers of small mitochondria in the early spermatid. Unlike other sperm so far reported, the Nebenkern goes through a stage where the mitochondrial material forms a tube, before producing the asymmetrically-sized mitochondrial derivatives (Fig. 9, *arrowed*). The association between the larger mitochondrial derivative and the nucleus is evident from the earliest stages of nuclear shape change (Fig. 10). Mitochondrial material

appears to be present at the locus of this change.

The centriolar adjunct in the adult sperm is positioned between the smaller mitochondrial derivative and the nucleus (Fig. 1). During development there is a close association between the centriolar adjunct and the developing axoneme (Fig. 11). In the cells where with multiple axonemes additional centriolar adjuncts are also present (Fig. 12) indicative of the close association between the two organelles.

*Nucleus.*—Axoneme development commences before nuclear elongation which follows a similar pattern to that described for the braconid *Aleiodes*, with the formation of lateral plates (Fig. 10) opposite aggregations of dense chromatin strands (*cf* Newman & Quicke 1998).

*Acrosome.*—The acrosome forms from fusion of small vesicles at the posterior

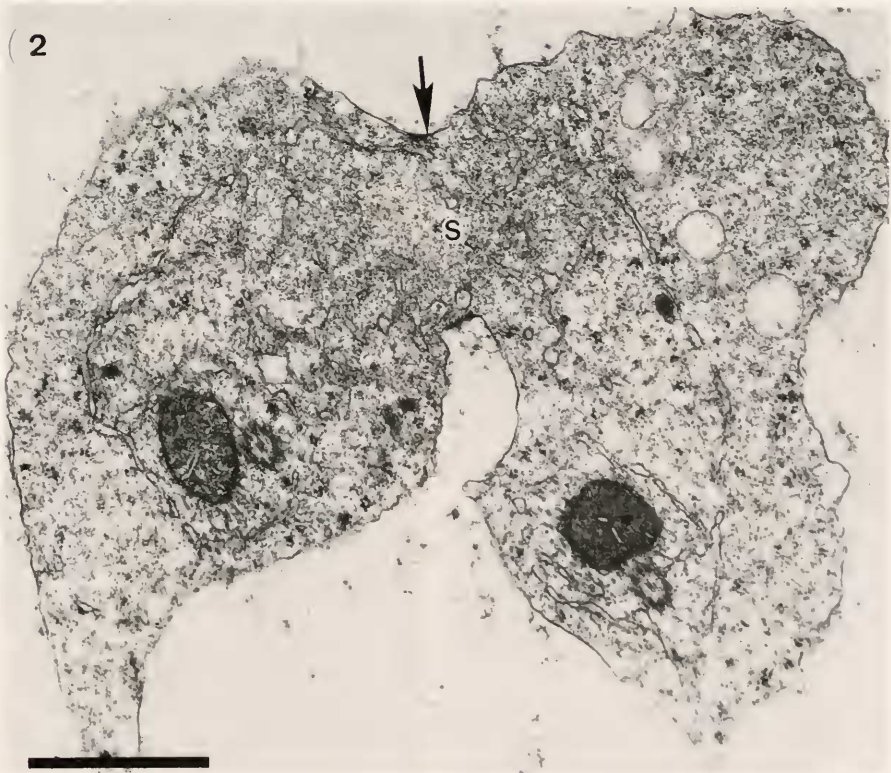
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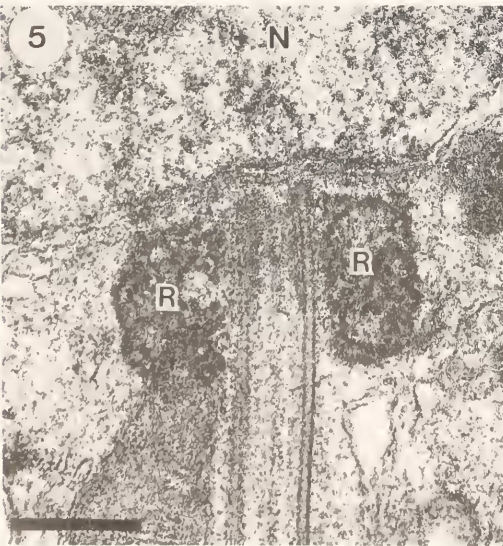
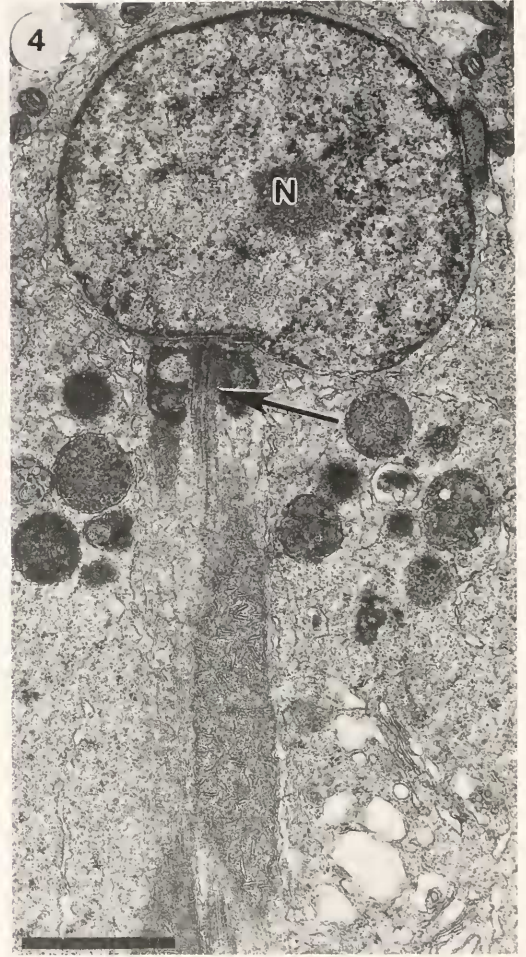
Figs. 1–2. Mature sperm and early spermiogenesis of *Megalyra*: 1, transversely sectioned vas deferens with mature sperm with axonemes (A), nucleus (N), asymmetrically sized mitochondrial derivatives (M)—including some with only one mitochondrial derivative (*arrowed*)—and centriolar adjunct (C) in some sections where it occupies the position of the smaller mitochondrial derivative just posterior to the nucleus (scale bar = 100 nm); 2, secondary spermatocytes forming a syncytium (S) because of incomplete cytokinesis following the earlier mitotic division, the thickened membrane (*arrowed*) indicating intercellular bridges (scale bar = 0.5  $\mu$ m).

Figs. 3–6. Various stages in spermiogenesis of *Megalyra*: 3, transverse section of the tail portion of mature sperm illustrating the 9 + 9 + 2 arrangement of microtubules, with accessory filaments evident, particularly linking the central pair of microtubules (*arrow*), and with two deltoid bodies (D) in association with the mitochondrial derivatives (scale bar = 100 nm); 4, early spermatid illustrating the growth of the axoneme (*arrowed*) from a position at the posterior pole of the nucleus (N) (scale bar = 500 nm); 5, early spermatid showing ring centriole (R) surrounding the basal body of the developing axoneme (scale bar = 250 nm); 6, during development many cell sections show multiple axoneme profiles (A), closer than would be expected if they represented sections through a single convoluted structure—note disruption of one of the profiles (*arrowed*) (scale bar = 100 nm).

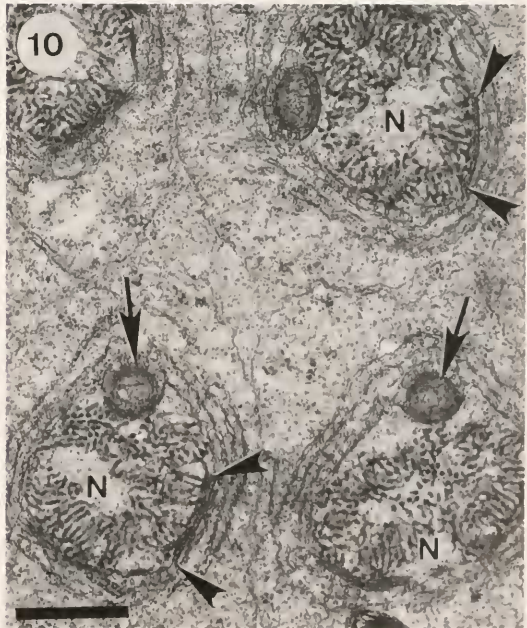
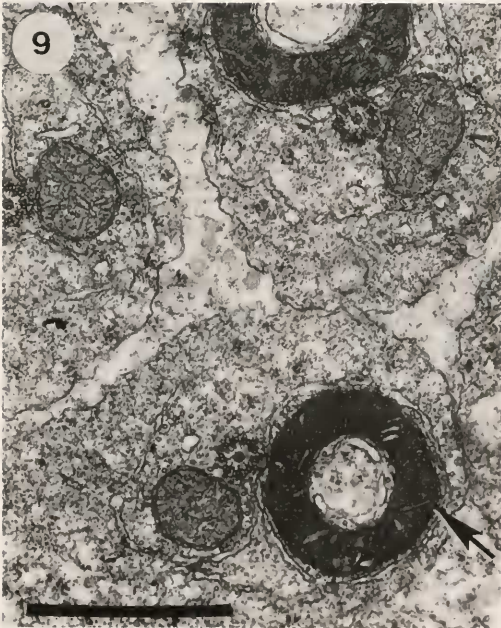
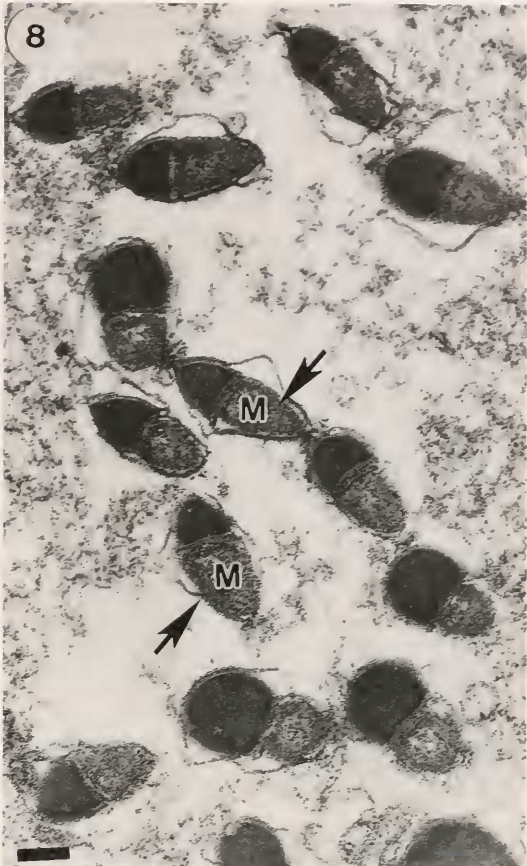
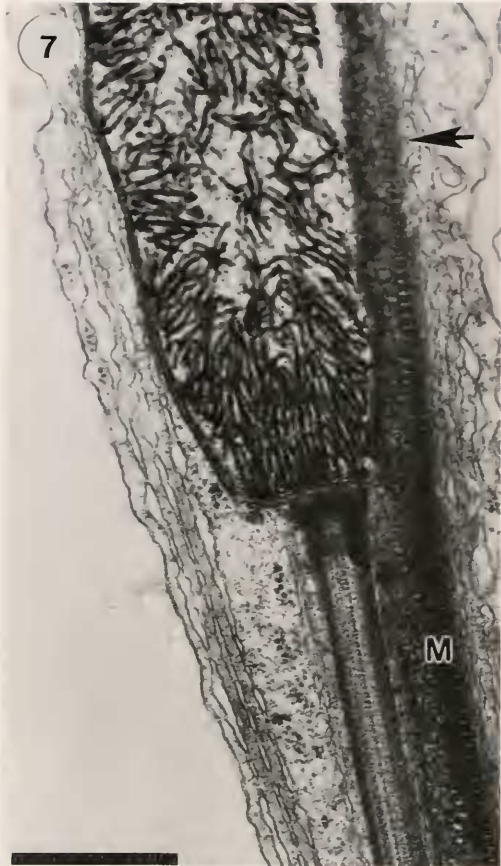
Figs. 7–10. Various stages in spermiogenesis of *Megalyra*: 7, longitudinal section of the sperm head piece with the larger mitochondrial derivative (M) extending from the tail portion, where it is found adjacent to the axoneme, and then running parallel to the nucleus (*arrowed*) (scale bar = 0.5  $\mu$ m); 8, transverse section through mature spermatozoan illustrating differences in the size of the nucleus (N) and of the large mitochondrial derivative (M) in the head portion of the sperm in the region of overlap between the two (scale bar = 100 nm); 9, transverse section of early spermatid illustrating a stage during formation of the mitochondrial derivatives when the Nebenkern becomes tubular (*arrowed*) (scale bar = 1  $\mu$ m); 10, one mitochondrial derivative (*arrowed*) lies partly in a groove along the nucleus during earlier stages of development—note also the lateral plates (between arrowheads) which appear to be anchor sites for condensed chromatin during nuclear shape change (scale bar = 0.5  $\mu$ m).



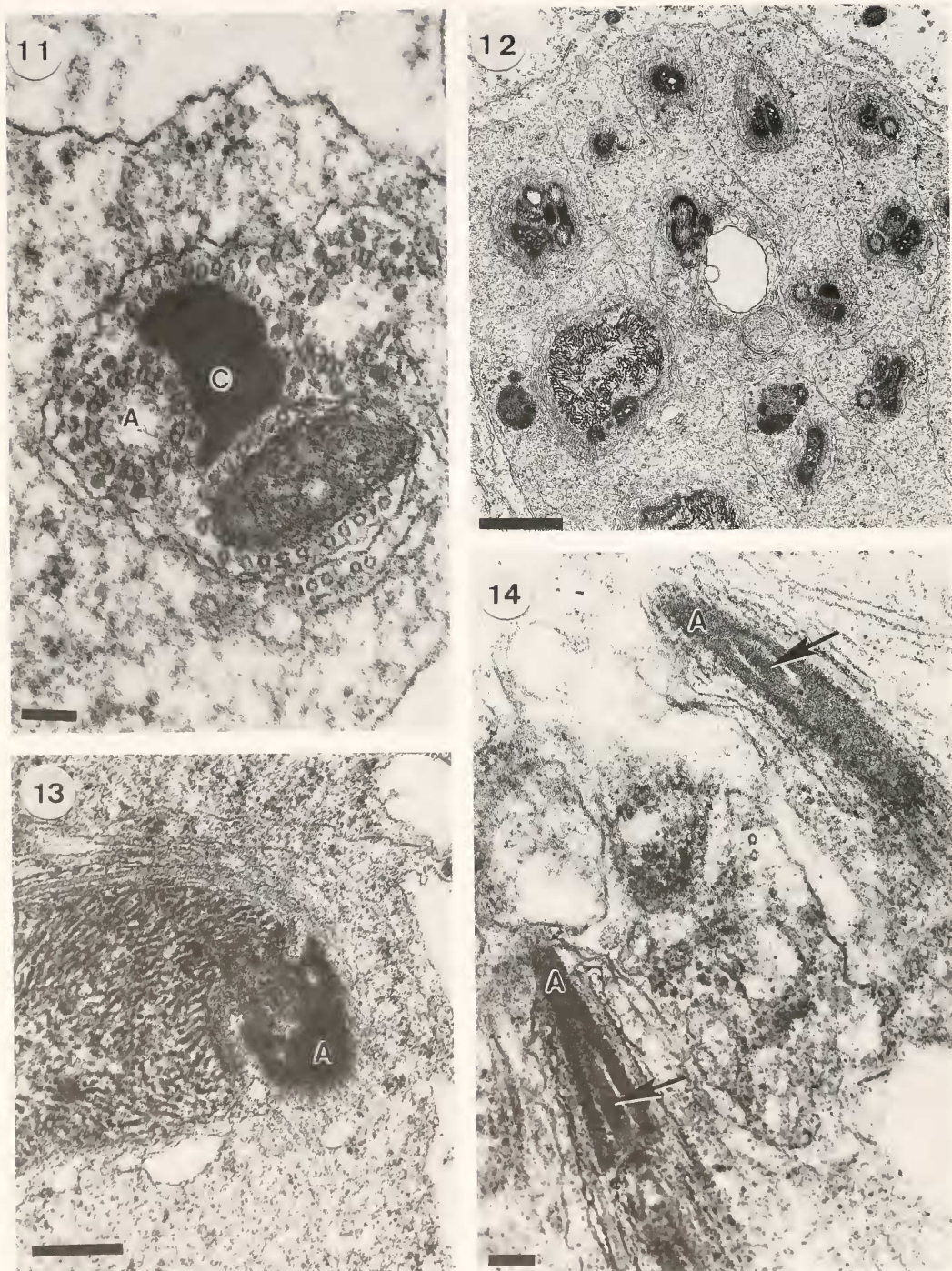












Figs. 11-14. Various stages in spermiogenesis of *Megalyra*: 11, transverse section of spermatid illustrating how the centriolar adjunct (C) is closely associated with the axoneme (A) during development (scale bar = 100 nm); 12, developing cyst of spermatids showing that cells with multiple axonemes have accompanying centriolar adjuncts (scale bar = 1  $\mu$ m); 13, during development the acrosome (A) is formed from the fusion of small vesicles and becomes positioned at the anterior pole of the nucleus (scale bar = 0.5  $\mu$ m); 14, acrosome in longitudinal section (A) covering the perforatorium (arrowed) (scale bar = 100 nm).

pole of the nucleus (Fig. 13). Although it develops a perforatorium (Fig. 14, *arrow*), the acrosome is small and ill-developed in comparison with the rest of the sperm and was very hard to find despite searching hundreds of transverse sections.

## DISCUSSION

The presence of two markedly differently sized mitochondrial derivatives has been reported in other parasitic wasps' spermatozoa, e.g. *Leptopilina*, which belongs to the only distantly related superfamily Cynipoidea (Newman and Quicke 1999b). We have also found a similar arrangement in the sperm of the xyelid sawfly, *Xyela julii* (Newman and Quicke 1999a). Whether this feature could be considered 'primitive' is doubtful given that other sawflies (*Cephalcia* of the Pamphiliidae and *Tremex* of the Siricidae) have equally sized mitochondrial derivatives.

The large length of overlap between one of the mitochondrial derivatives and the nucleus has only been found in sperm of one other parasitic wasp, the distantly related chalcidoid wasp genus *Trichogramma* (Lingmei & Dunsu 1987). The cynipoid, *Leptopilina*, which also has asymmetric sized mitochondrial derivatives, also has an overlap of nucleus and mitochondrial derivative, but over a much smaller distance (Newman & Quicke 1999b).

This intimate relationship of mitochondrial material and nucleus exists from an early stage of spermiogenesis. The mitochondrial material is found at the focus of the nuclear shape change which occurs during cell elongation, where the nucleus curves into a horse-shoe shape, a shape change similar to that found in *Aleiodes* (Newman & Quicke 1998). One aspect of development which has not been previously reported is the occurrence of tubular elements in the Nebenkern after fusion of the small mitochondria of the early spermatid. This is probably an apomorphic character state and it may be of potential

phylogenetic significance within the Evaniomorpha.

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